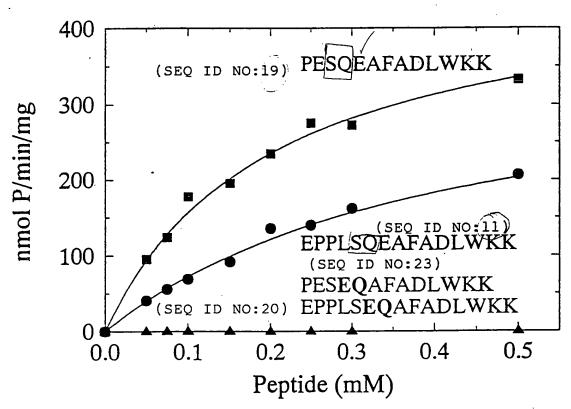
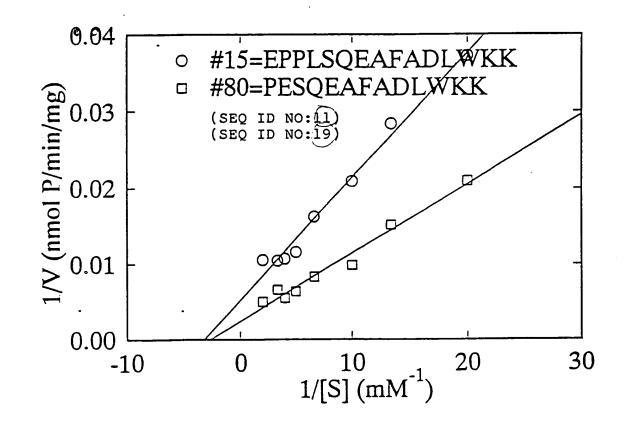
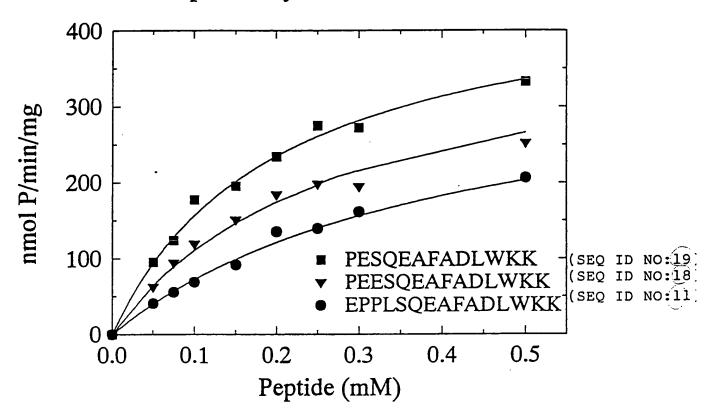
Phosphorylation of Synthetic Peptides by Human DNA-PK



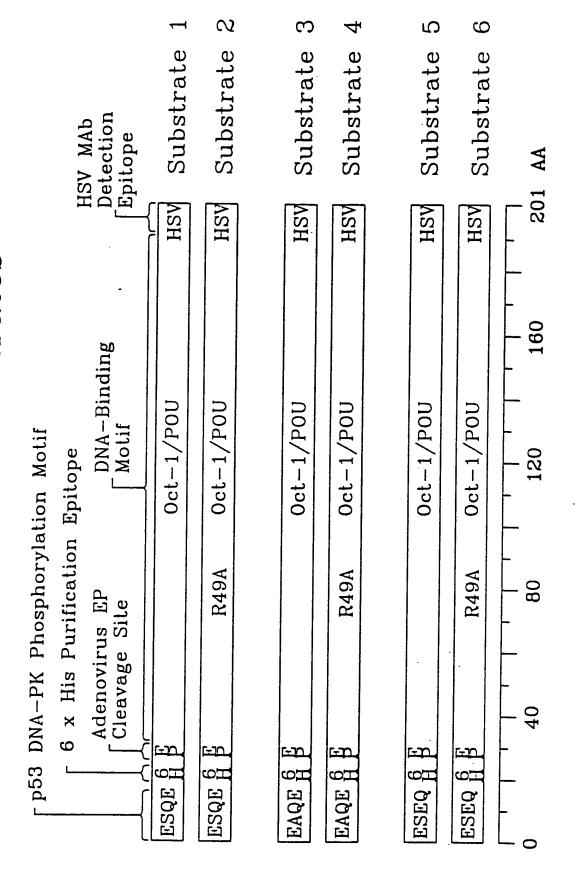
Phosphorylation of Synthetic Peptides by Purified Human DNA-PK



Phosphorylation of Synthetic Peptides by Human DNA-PK



Artificial DNA-PK Substrates



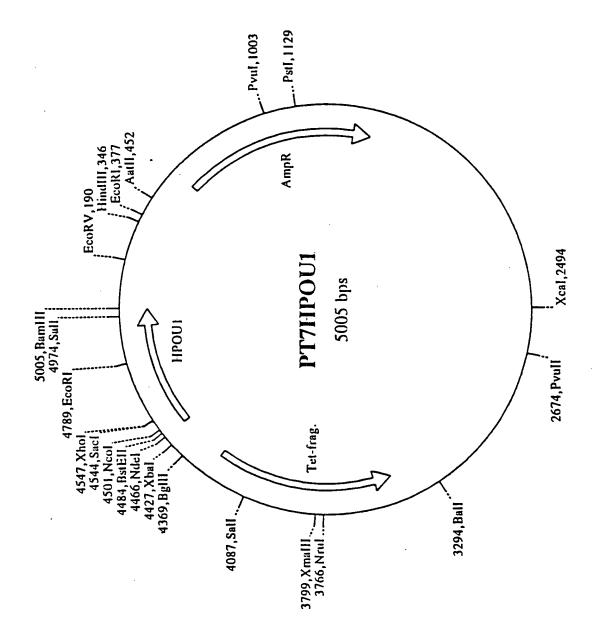


FIGURE 5B

Expressed Prot in Product of pT7HPOU1

pT7HPOU1: Expression Vector for Human Oct-1 POU Domain with His6

leader

T7HPOU1 5005 bases, circular

Lab Strain: #236 = pT7HPOU1/DH5[alpha] Lab Strain: #237 = pT7HPOU1/BL21(DE3)

Plasmid Construction:

Vector: pT7HIS2 (pET-3 with His6 leader and T7 gene 2.5)

Cut with Nco I and BamH I

Insert: POU domain from pET11c-OCT1POU (CWA Strain #234) from

Winship Herr, Cold Spring Harbor Laboratory. POU domain DNA was made by PCR using primers #761 and #430 (pBR322 EcoR I site). PCR fragment was cut with NcoI and BamHI, purified, and inserted in similarly cut

pT7HIS2 vector (also called pT7AdEP-DBP).

PREDICTED PROTEIN SEQUENCE OF EXPRESSION PRODUCT Segment: 4469-5005

(SEQ ID NO:59)

Composition

8	Ala	7	Gln	18	Leu	18	Ser
12	Arg	17	Glu	15	Lys	9	Thr
11	Asn	11	Gly	8	Met	2	Trp
6	Asp	6	His	8	Phe	1	Tyr
2	Cys	9	Ile	6	Pro	4	Val

Mol. wt. unmod. chain = 20,352 Number of residues = 178

Met Ala Ser Met Thr Gly His His His His His Gly Met Ser Gly
1 5 15

Gly Met Glu Glu Pro Ser Asp Leu Glu Glu Leu Glu Gln Phe Ala Lys
20 25 30

Thr Phe Lys Gln Arg Arg Ile Lys Leu Gly Phe Thr Gln Gly Asp Val

Gly Leu Ala Met Gly Lys Leu Tyr Gly Asn Asp Phe Ser Gln Thr Thr 50 55 60

. . . .

FIGURE 5B (Continued)

(SEQ ID NO:59)

Ile Ser Arg Phe Glu Ala Leu Asn Leu Ser Phe Lys Asn Met Cys Lys 70 75 Leu Lys Phe Leu Leu Glu Lys Trp Leu Asn Asp Ala Glu Asn Leu Ser Ser Asp Ser Ser Leu Ser Ser Pro Ser Ala Leu Asn Ser Pro Gly Ile 105 Glu Gly Leu Ser Arg Arg Arg Lys Lys Arg Thr Ser Ile Glu Thr Asn 125 115 Ile Arg Val Leu Glu Lys Ser Phe Leu Glu Asn Gln Lys Pro Thr Ser 135 Glu Glu Ile Thr Met Ile Ala Asp Gln Leu Asn Met Glu Lys Glu Val 150 155 Ile Arg Val Trp Phe Cys Asn Arg Arg Gln Lys Glu Lys Arg Ile Asn 165 170 175 Pro

Reference: Anderson, C. W., and S. P. Lees-Miller. 1992. The nuclear serine/threonine protein kinase DNA-PK. Crit. Rev. Eukaryotic Gene Express. 2, 283-314.

Figure 5C

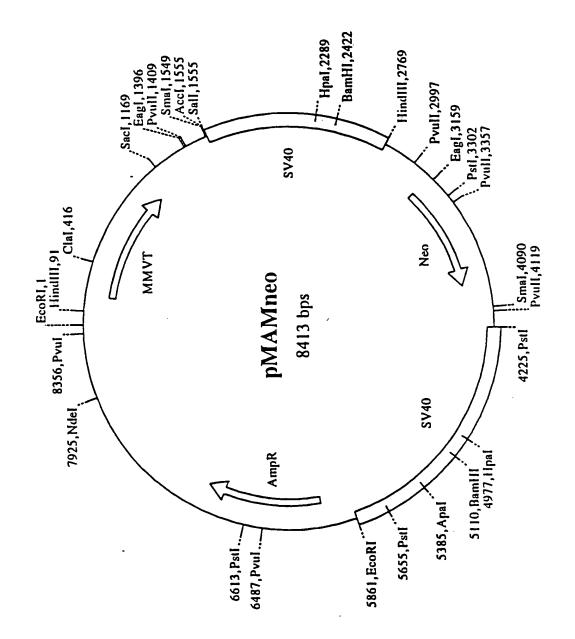
NUCLEOTIDE SEQUENCE OF pT7HPOU1

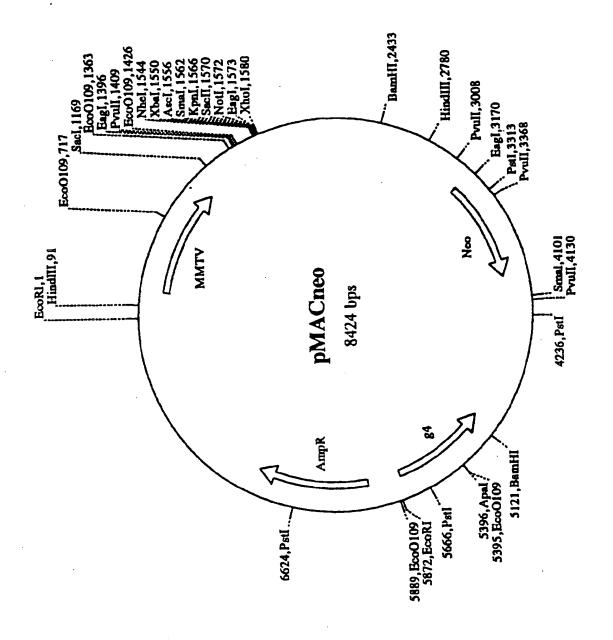
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TGCGCGGAAC	CCCTATTTGT	TTATTTTTCT	AAATACATTC	AAATATGTAT	CCGCTCATGA	540
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TCGAACTGGA	TCTCAACAGC	GGTAAGATCC	TTGAGAGTTT	TCGCCCCGAA	GAACGTTTTC	780
CAATGATGAG	CACTTTTAAA	GTTCTGCTAT	GTGGCGCGGT	ATTATCCCGT	GTTGACGCCG	840
GGCAAGAGCA	ACTCGGTCGC	CGCATACACT	ATTCTCAGAA	TGACTTGGTT	GAGTACTCAC	900
CAGTCACAGA	AAAGCATCTT	ACGGATGGCA	TGACAGTAAG	AGAATTATGC	AGTGCTGCCA	960
TAACCATGAG	TGATAACACT	GCGGCCAACT	TACTTCTGAC	AACGATCGGA	GGACCGAAGG	1020
AGCTAACCGC	TTTTTTGCAC	AACATGGGGG	ATCATGTAAC	TCGCCTTGAT	CGTTGGGAAC	1080
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TAATAGACTG	GATGGAGGCG	GATAAAGTTG	CAGGACCACT	TCTGCGCTCG	GCCCTTCCGG	1260
CTGGCTGGTT	TATTGCTGAT	AAATCTGGAG	CCGGTGAGCG	TGGGTCTCGC	GGTATCATTG	1320
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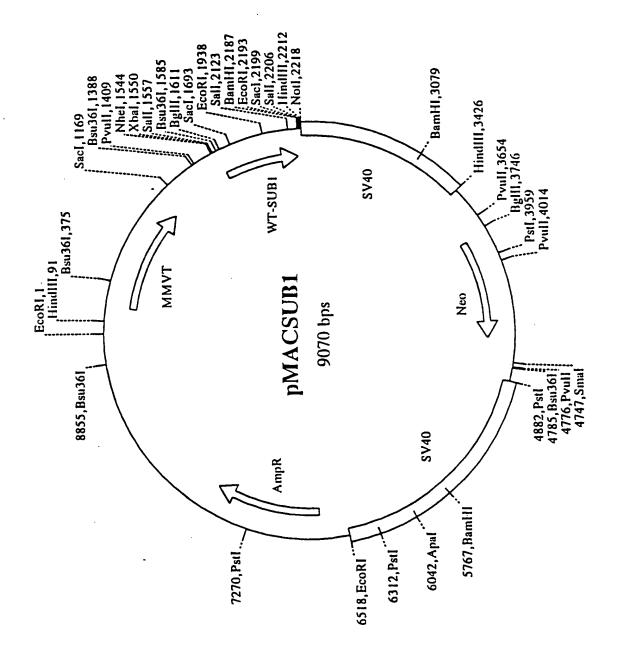
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GTC	cccc	GC ·	CACG	GGGC	CT G	CCAC	CATAC	c cci	ACGC	CGAA	ACA	AGCG	CTC I	ATGA	GCCCGA	4260
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CAC	CAT	CAC	CAT	GGT	ATG	AGC	GGC	GGC	ATG	GAG	GAG	CCC	AGT	GAC	CTT	4540
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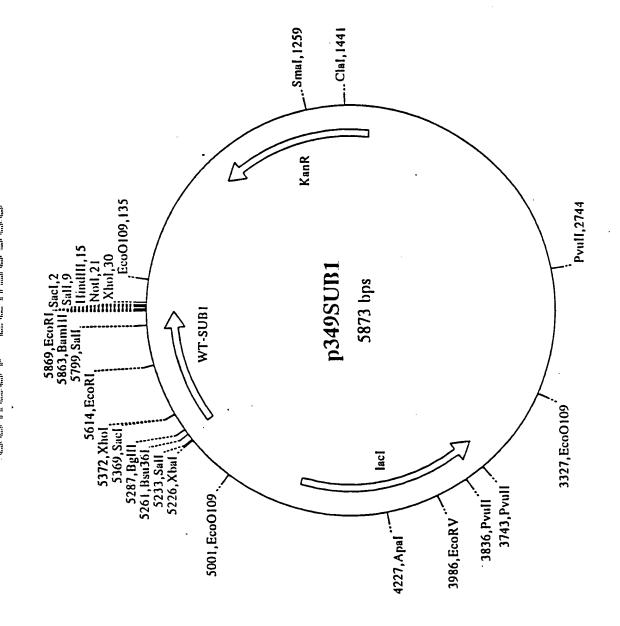


FIGURE 8B

Wild-Type Artificial DNA-PK Substrat 1

Lab Stain: #349 = p349SUB1 in DH5[alpha] Lab Strain #351 = p349SUB1 in BL21(DE3)

Plasmid Construction:

VECTOR: p410 = derivative pET-28a (Novagen) without BglII site INSERT: Substrate encoding XbaI-BamHI fragment was excised from

p345 with XbaI and BamHI and cloned into XbaI and BamHI

22 Leu

16 Lys

18 Ser

9 Thr

95

110

cleaved p410.

8 Ala

85

100

12 Arg

ANTIBIOTIC SELECTION: 50 ug/ml Kanamycin

PREDICTED SEQUENCE POUSUB1 ARTIFICIAL DNA-PK SUBSTRATE

Segment: 5258-5860

(SEQ ID NO. 61)

Composition

9 Gln

24 Glu

		1	1 Asi 9 Asi 2 Cy	p		11 G 6 H 9 I	is		્9 :	Met Phe Pro		3 1	Trp Tyr Val		
Mol. wt. unmod. chain = 23,126									Number of residues = 201						L
Met 1	Pro	Glu	Glu	Ser 5	Gln	Glu	Thr	Phe	Glu 10	Asp	Leu	Trp	Lys	Leu 15	Leu
Pro	Gly	His	His 20	His	His	His	His	Gly 25	Met	Ser	Gly	Gly	Met 30	Glu	Glu
Pro	Ser	Asp 35	Leu	Glu	Glu	Leu	Glu 40	Gln	Phe	Ala	Lys	Thr 45	Phe	Lys	Gln
Arg	Arg 50	Ile	Lys	Leu	Gly	Phe 55	Thr	Gln	Gly	Asp	Val 60	Gly	Leu	Ala	Met
Gly 65	Lys	Leu	Tyr	Gly	Asn 70	Asp	Phe	Ser	Gln	Thr 75	Thr	Ile	Ser	Arg	Phe 80
Glu	Ala	Leu	Asn	Leu	Ser	Phe	Lys	Asn	Met	Cys	Lys	Leu	Lys	Pro	Leu

Leu Glu Lys Trp Leu Asn Asp Ala Glu Asn Leu Ser Ser Asp Ser Ser

105

90

FIGURE 8B (Continued)

(SEQ ID NO:61)

```
Leu Ser Ser Pro Ser Ala Leu Asn Ser Pro Gly Ile Glu Gly Leu Ser
        115
                             120
Arg Arg Arg Lys Lys Arg Thr Ser Ile Glu Thr Asn Ile Arg Val Ala
                         135
                                             140
Leu Glu Lys Ser Phe Leu Glu Asn Gln Lys Pro Thr Ser Glu Glu Ile
                    150
                                         155
Thr Met Ile Ala Asp Gln Leu Asn Met Glu Lys Glu Val Ile Arg Val
                165
                                                         175
Trp Phe Cys Asn Arg Arg Gln Lys Glu Lys Arg Ile Asn Pro Gln Pro
            180
                                 185
Glu Leu Ala Pro Glu Asp Pro Glu Asp
        195
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NOTES:
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Figure 8C

NUCLEOTIDE SEQUENCE OF p349SUB1

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CACCTGTCCT	ACGAGTTGCA	TGATAAAGAA	GACAGTCATA	AGTGCGGCGA	CGATAGTCAT	3600
GCCCGCGCC	CACCGGAAGG	AGCTGACTGG	GTTGAAGGCT	CTCAAGGGCA	TCGGTCGAGA	3660
TCCCGGTGCC	TAATGAGTGA	GCTAACTTAC	ATTAATTGCG	TTGCGCTCAC	TGCCCGCTTT	3720
CCAGTCGGGA	AACCTGTCGT	GCCAGCTGCA	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	3780
CGGTTTGCGT	ATTGGGCGCC	AGGGTGGTTT	TTCTTTTCAC	CAGTGAGACG	GGCAACAGCT	3840
GATTGCCCTT	CACCGCCTGG	CCCTGAGAGA	GTTGCAGCAA	GCGGTCCACG	CTGGTTTGCC	3900
CCAGCAGGCG	AAAATCCTGT	TTGATGGTGG	TTAACGGCGG	GATATAACAT	GAGCTGTCTT	3960
CGGTATCGTC	GTATCCCACT	ACCGAGATAT	CCGCACCAAC	GCGCAGCCCG	GACTCGGTAA	4020
TGGCGCGCAT	TGCGCCCAGC	GCCATCTGAT	CGTTGGCAAC	CAGCATCGCA	GTGGGAACGA	4080
TGCCCTCATT	CAGCATTTGC	ATGGTTTGTT	GAAAACCGGA	CATGGCACTC	CAGTCGCCTT	4140

CCCGTTCCGC	TATCGGCTGA	ATTTGATTGC	GAGTGAGATA	TTTATGCCAG	CCAGCCAGAC	4200
GCAGACGCGC	CGAGACAGAA	CTTAATGGGC	CCGCTAACAG	CGCGATTTGC	TGGTGACCCA	4260
ATGCGACCAG	ATGCTCCACG	CCCAGTCGCG	TACCGTCTTC	ATGGGAGAAA	ATAATACTGT	4320
TGATGGGTGT	CTGGTCAGAG	ACATCAAGAA	ATAACGCCGG	AACATTAGTG	CAGGCAGCTT	4380
CCACAGCAAT	GGCATCCTGG	TCATCCAGCG	GATAGTTAAT	GATCAGCCCA	CTGACGCGTT	4440
GCGCGAGAAG	ATTGTGCACC	GCCGCTTTAC	AGGCTTCGAC	GCCGCTTCGT	TCTACCATCG	4500
ACACCACCAC	GCTGGCACCC	AGTTGATCGG	CGCGAGATTT	AATCGCCGCG	ACAATTTGCG	4560
ACGGCGCGTG	CAGGGCCAGA	CTGGAGGTGG	CAACGCCAAT	CAGCAACGAC	TGTTTGCCCG	4620
CCAGTTGTTG	TGCCACGCGG	TTGGGAATGT	AATTCAGCTC	CGCCATCGCC	GCTTCCACTT	4680
TTTCCCGCGT '	TTTCGCAGAA	ACGTGGCTGG	CCTGGTTCAC	CACGCGGGAA	ACGGTCTGAT	4740
AAGAGACACC	GGCATACTCT	GCGACATCGT	ATAACGTTAC	TGGTTTCACA	TTCACCACCC	4800
TGAATTGACT	CTCTTCCGGG	CGCTATCATG	CCATACCGCG	AAAGGTTTTG	CGCCATTCGA	4860
TGGTGTCCGG	GATCTCGACG	CTCTCCCTTA	TGCGACTCCT	GCATTAGGAA	GCAGCCCAGT	4920
AGTAGGTTGA	GGCCGTTGAG	CACCGCCGCC	GCAAGGAATG	GTGCATGCAA	GGAGATGGCG	4980
CCCAACAGTC	CCCCGGCCAC	GGGGCCTGCC	ACCATACCCA	CGCCGAAACA	AGCGCTCATG	5040
AGCCCGAAGT (GGCGAGCCCG	ATCTTCCCCA	TCGGTGATGT	CGGCGATATA	GGCGCCAGCA	5100
ACCGCACCTG	TGGCGCCGGT	GATGCCGGCC	ACGATGCGTC	CGGCGTAGAG	GATCGAGATC	5160
GATCTCGATC	CCGCGAAATT	AATACGACTC	ACTATAGGGG	AATTGTGAGC	GGATAACAAT	5220
TCCCCTCTAG	AAGTCGACTT	TAAGAAGGAG	TACCAAG ATO	CCT GAG GA	AA AGT CAG	5275
GAG ACA TTC	GAA GAT CT	'A TGG AAA (CTA CTT CCT	GGT CAC CAC	C CAC CAT	5323
CAC CAT GGT	ATG AGC GG	C GGC ATG	GAG GAG CCC	AGT GAC CT	r GAG GAG	5371
CTC GAG CAG	TTT GCC AA	G ACC TTC A	AAA CAA AGA	CGA ATC AAA	A CTT GGA	5419
TTC ACT CAG	GGT GAT GT	T GGG CTC	GCT ATG GGG	AAA CTA TAT	GGA AAT	5467

GAC	110	AGC	CAA	ACI	MCC	AIC	ICI	CGA	TTT	GAA	GCC	TTG	AAC	CTC	AGC	5515
TTT	AAG	AAC	ATG	TGC	AAG	TTG	AAG	CCA	CTT	TTA	GAG	AAG	TGG	CTA	AAT	5563
GAT	GCA	GAG	AAC	CTC	TCA	TCT	GAT	TCG	TCC	CTC	TCC	AGC	CCA	AGT	GCC	5611
CTG	AAT	TCT	CCA	GGA	ATT	GAG	GGC	TTG	AGC	AGG	CGC	CGT	AAG	AAA	CGC	5659
ACC	AGC	ATA	GAG	ACC	AAC	ATC	CGT	GTG	GCC	TTA	GAG	AAG	AGT	TTC	TTG	5707
GAG	AAT	CAA	AAG	CCT	ACC	TCG	GAA	GAG	ATC	ACT	ATG	ATT	GCT	GAT	CAG	5755
CTC	AAT	ATG	GAA	AAA	GAG	GTG	ATT	CGT	GTT	TGG	TTC	TGT	AAC	CGT	CGA	5803
CAG	AAA	GAA	AAA	AGA	ATC	AAC	CCA	CAG	CCA	GAA	CTC	GCC	CCG	GAA	GAC	5851
ccc	GAG	GAT	TAGO	ATC	CGA A	\TT										5873

Phosphorylation of Recombinant Substrates by Purified Human DNA-PK

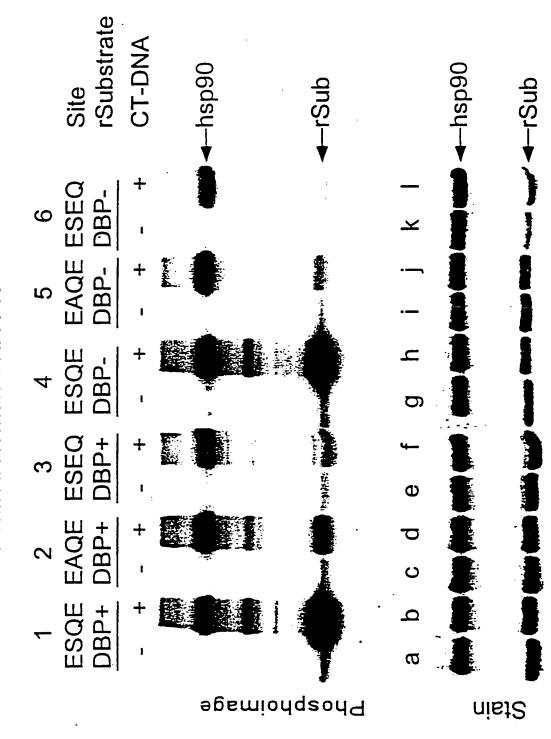


FIGURE 9

Isoelectrofocusing Analysis of Recombinant DNA-PK Substrates

